Journal of Chromatography, 368 (1986) 381–386
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 915

Note

Derivatization of trichothecenes and water treatment of their trimethylsilyl ethers in an anhydrous apolar solvent

A. F. RIZZO*, L. SAARI and E. LINDFORS
National Veterinary Institute, P. Box 368, 00101 Helsinki (Finland)
(First received May 20th, 1986; revised manuscript received July 7th, 1986)

The Fusarium mycotoxins, better known as trichothecenes are natural contaminants, often found in cereals, animal feed and human food[1–3]. The trichothecenes have been used in biological warfare [4,5] and their toxicity toward animals and humans is well documented[5–8].

Several different analytical techniques have been used for their investigation, but the most sensitive and specific ones are gas chromatography with electron-capture detection (GC–ECD) and gas chromatography–mass spectrometry (GC–MS)[1,3,9]. The most common methods of derivatization are silylation, which transforms the trichothecenes into trimethylsilyl ethers (TMSE), trifluoroacetylation and heptafluorobutyrylation which convert the mycotoxins into trifluoroacetyl and heptafluorobutyryl esters respectively. The last two types of compounds give a very high ECD response, but the impurities which usually accompany the trichothecenes are derivatized too, and produce very complicated gas chromatograms. This makes it very difficult, if not impossible, to identify the trichothecenes. Silylation is often preferred because it gives much less complicated gas chromatograms.

In our study we have used trimethylsilylimidazole, with and without trimethylchlorosilane, for the silylation since from our experience and on the basis of the literature it is the most effective reagent for the silylation of trichothecenes[2,10]. The aim of this work was to improve the detectibility of six of the most important trichothecenes by performing selective hydrolysis of the reagent, after silylation, in order to eliminate its interfering effect on the products of derivatization during GC.

EXPERIMENTAL

Materials

Trimethylsilylimidazole (TSIM) and trimethylchlorosilane (TMCS) were supplied by Pierce (Eurochemie, Rotterdam, The Netherlands), 4-deoxynivalenol (DON), diacetoxyscirpenol (DAS), fusarenon-X (F-X) and nivalenol (NV) were purchased from Myco-Lab (Chesterfield, MO, U.S.A.), T-2 and HT-2 toxins were from Sigma (St. Louis, MO, U.S.A.) and hexane and ethyl acetate were analytical grade from E. Merck (Darmstadt, F.R.G.).

0021-9673/86/$03.50

Derivatization

A stock solution containing DON, F-X, NV and DAS was prepared in dichloromethane at a concentration of 0.5 μg/ml, except for DAS which was 1.4 μg/ml (solution A). The concentration of the T-2 and HT-2 toxin solution was 17 μg/ml, also in dichloromethane (solution B).

The silylating agents were TSIM (reagent I) and a mixture of TSIM, TMCS and ethyl acetate (1:0.2:9) (reagent II) freshly prepared each day, according to Tanaka *et al.*[11]. An 150-μl volume of stock solution A and 25 μl of stock solution B were dispended in a 3-ml vial (four replicate). The solvent was evaporated by a stream of nitrogen; three vials were supplemented with 100 μl of reagent I, and one vial with 100 μl of reagent II. After flushing with nitrogen, the PTFE-lined screw-caps were closed tightly and the samples allowed to react at room temperature for 15 min (reagent II) and 1 h at 60, 80 and 100°C (reagent I). It should be noted that if the reagent is rather old or kept in unsealed containers it gives a lower yield of TMSE.

Water treatment of the trimethylsilyl ethers

After derivatization, the vials were cooled (reagent I). The samples were supplemented with 1 ml of solvent (hexane for reagent I; hexane or ethyl acetate for reagent II), quickly mixed, 1 ml of distilled water was added and shaken for 10 s on a Vortex mixer. After standing for 30 s, the solvent (upper phase) was transferred to another vial and stored in a freezer till analysis.

Analysis

GC analysis of the samples was performed on a Varian gas chromatograph (Model 3700) equipped with ECD (^{63}Ni foil) and a fused-silica column (25 m × 0.32 mm I.D.) coated with 0.2-μm Noribond OV-1 bonded phase (Nordion Oy, Helsinki, Finland). The carrier gas was helium at 10 p.s.i.g. (about 1.6 ml/min), and the make-up gas was nitrogen at a flow-rate of 30 ml/min. A 1-μl volume of the sample was injected in the splitless mode. The temperatures of the injector and detector were 250 and 300°C respectively. The oven temperature was 60°C for 1 min, then increased to 180°C at 40°C/min and finally to 250°C at 5°C/min. An electronic integrator Varian (Model 4270) was connected to the gas chromatograph for quantitative analysis of the trichothecenes.

The identity of the TMSEs was confirmed using a DANI HR gas chromatograph (Model 3800) together with a JEOL DX-300 mass spectrometer, operated in the electron-impact mode at 30 eV and equipped with a Texas Instrument 980 B data system. The same column and GC conditions as described above were used. The interface temperature was 250°C, the ionization current 300 μA and the ion-source temperature 180°C. The sample was concentrated under nitrogen to a volume of 50–70 μl, and 3 μl were injected in the splitless mode. The scanning range was m/z 70–600.

RESULTS AND DISCUSSION

In the normal procedure, the GC analysis of trichothecenes is performed by injecting the derivatized sample in the presence of the silylating agent, diluted in solvent or undiluted[3,10,11]. Fig. 1 shows the remarkable difference in intensity of the

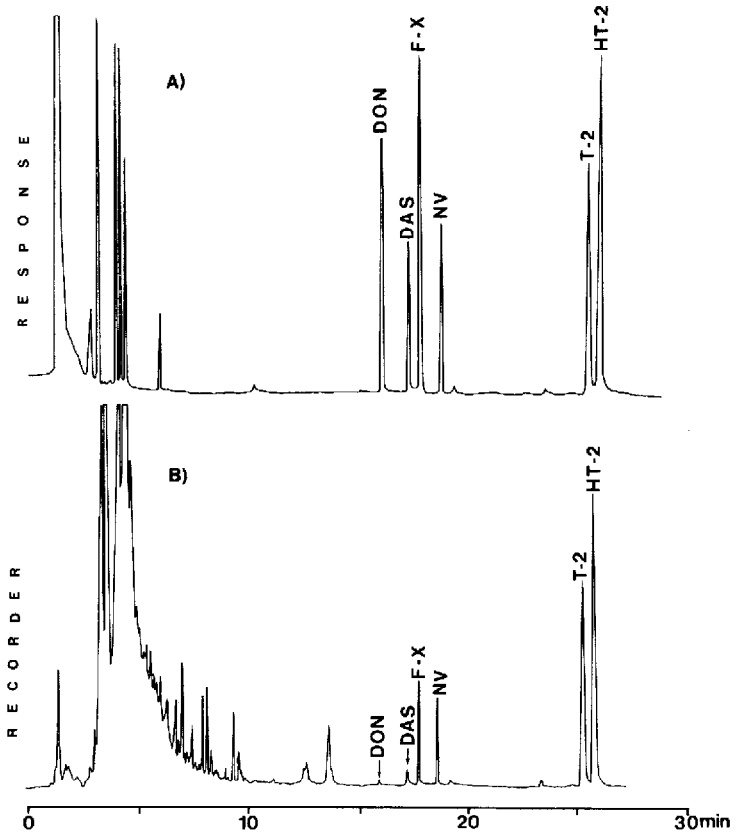

Fig. 1. Gas chromatograms of silylated trichothecenes. The same sample was injected after derivatization, without water treatment (B) and after treating it with water (A). The 25-m GC column was fused silica, coating OV-1. ECD, attenuation 512.

TABLE I

EFFECT OF THE WATER TREATMENT ON SOME TRICHOTHECENES DERIVATIZED WITH REAGENT II UNDER THE SAME CONDITIONS

The highest peak area is assumed to be 100.

Trichothecene	*Treated with water*	*Untreated*
DON	100	1.6
DAS	100	8.50
F-X	100	22.80
NV	100	37.50
T-2	100	89.50
HT-2	100	87.50

peaks of DON, DAS, F-X and NV when injected in the presence of the reagent and when the latter has been hydrolyzed and removed with water. Only in the cases of T-2 and HT-2 toxins is the amount of TMSEs practically the same. The values for the trichothecenes detected, after derivatization with 100 μl of reagent II and subsequent dilution in 1 ml of hexane, for sample injection before and after the water treatment, are compared in Table I. The two sets of results are about the same when the silylation is performed with reagent I. If, after derivatization, the sample is injected without dilution in the solvent but in the reagent itself, the results are even worse, since the only trichothecenes detected were T-2 and HT-2.

When a polar solvent such as ethyl acetate was added to the reacted sample and no water treatment was performed, T-2 and HT-2 were the only trichothecenes detected, although in smaller amounts than in the case of samples diluted in hexane. Higher dilution of the reacted sample was tested too (4 ml of hexane or ethyl acetate), but no trichothecene was detected in this case.

Moderate thermal treatment (60°C for 1 h) improves the yield of TMSEs when reagent I is used. Increasing the temperature (80 and 100°C) or prolonged reaction did not improve the result.

Evaporation of reagent I was also tested. After silylation, the reagent was evaporated under a stream of nitrogen with a Pasteur pipette placed about half a centimetre below the mouth of the reaction vial. After 2 h and 30 min the evaporation of TSIM, even if not complete, was terminated. The tip of the Pasteur pipette, which became coated with a white powder, was washed with 0.5 ml of hexane. A 1-μl volume of this solution was injected in the splitless mode. As is seen from Fig. 2, most of the trichothecenes were lost during evaporation of the reagent.

It can be concluded that, in the case of trichothecenes, after the silylation has been performed the reagent should be removed. This is also supported by the fact that, after the reaction, moderate dilution of the sample using an apolar solvent (hexane) gives a better result than the undiluted sample or if the sample is diluted in the reagent itself. In other words, after the trichothecenes have been converted into TMSEs, the smaller the amount of reagent injected together with the sample the

TABLE II

YIELD OF DERIVATIZATION, AS A PERCENTAGE, OF SOME TRICHOTHECENES SILYLATED WITH VARIOUS REAGENTS UNDER VARIOUS CONDITIONS, FOLLOWED BY HEXANE DILUTION AND WATER TREATMENT

The values are expressed as in Table I. Reagents: I = TSIM; II = TSIM–TMCS–ethyl acetate.

Trichothecene	*Reagent I*		*Reagent II*
	Room temp., 1 h	*60°C, 1 h*	*Room temp., 30 min*
DON	56	100	91
DAS	35	97	100
F-X	57	100	93
NV	65	100	78
T-2	46	100	80
HT-2	62	100	78

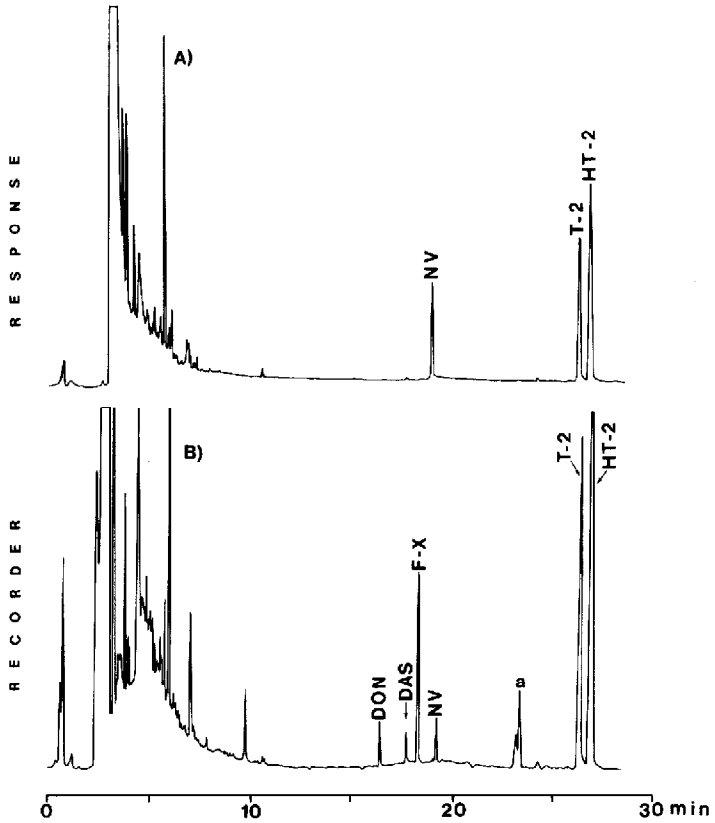

Fig. 2. Gas chromatograms of the residual silylated trichothecenes remaining in the reaction vial after evaporating the reagent at room temperature under nitrogen (A), and of the trichothecenes condensed on the pipette tip used for evaporating the reagent (B). GC conditions as in Fig. 1, but with signal attenuation 128. a = Artifact.

better is the end result. Evaporation of the reagent is not suitable for the above-mentioned reasons, but treatment with water is very simple, fast and effective. This also avoids the problem of the deterioration of the stationary phase caused by the injected reagent[10].

Another important advantage of the water treatment is the very effective removal from ordinary samples (cereals, feeds, etc.) of coloured pigments and other colourless compounds which often accompany the trichothecenes up to the very last step of the analytical procedure. Water selectively hydrolyzes several of the silylated impurities, possibly present, which are then extracted, leaving the trichothecene trimethylsilyl ethers largely or totally unaltered in the hexane phase.

REFERENCES

1 H. K. Abbas, C. J. Mirocha and W. T. Shier, *Appl. Environ. Microbiol.*, 48 (1984) 654.
2 E. Karppanen, A. F. Rizzo, S. Berg, E. Lindfors and R. Aho, *J. Sci. Agric. Soc. Finl.*, 57 (1985) 195.
3 J. Gilbert, M. J. Shepherd and J. R. Startin, *J. Sci. Food Agric.*, 34 (1983) 86.

4 R. T. Rosen and J. D. Rosen, *Biomed. Mass Spectrom.*, 9 (1982) 443.
5 C. J. Mirocha, R. A. Pawlosky, K. Chatteriee, S. Watson and Y. Hayes, *J. Assoc. Off. Anal. Chem.*, 66 (1983) 1485.
6 Y. Ueno, in Y. Ueno (Editor), *Historical Background of Trichothecene Problems*, Elsevier, Amsterdam, 1983, p. 1.
7 H. A. Poston, J. L. Coffin and G. F. Combs, Jr., *Aquatic Toxicol.*, 2 (1982) 79.
8 J. R. Bamburg and F. M. Strong, in S. Kadish (Editor), *Microbial Toxins*, Academic Press, New York, 1976, p. 401.
9 M. O. Moss and J. E. Smith (Editors), *Symposium of the British Mycological Society, London, September, 1982*, Cambridge Univ. Press, London, 1982, pp. 181–188.
10 J. Gilbert, J. R. Startin and C. Crews, *J. Chromatogr.*, 319 (1985) 376.
11 T. Tanaka, A. Hasegawa, Y. Matsuki, K. Ishii and Y. Ueno, *Food Additiv. Contam.*, 2 (1985) 125.